Lipid Peroxidation (MDA) Colorimetric Assay kit

Cat #: orb1173250 (manual)

Size: 48T / 96T

Microassay

Applicable samples: Serum, Plasma, Urine, Animal and Plant Tissues, Cells, Bacteria

Storage: Stored at 4℃ for 6 months

Assay Principle

Oxygen free radicals react on lipid unsaturated fatty acids to produce lipid peroxidation. The latter gradually breaks down into a series of complex compounds, including malondialdehyde (MDA). Lipid oxidation levels can be measured by detecting MDA levels. Lipid peroxidation may contribute to many diseases, including atherosclerosis, diabetes, and Alzheimer's disease. Lipid Peroxidation (MDA) Assay kit (Microassay) provides a convenient tool for detection of the malondialdehyde (MDA) present in a variety of samples. Malondialdehyde (MDA) can be condensed with thiobarbituric acid (TBA) under acidic and high temperature conditions to produce the brown-red trimethyltran (3,5, 5-trimethyloxazol-2, 4-dione) with a maximum absorption wavelength of 532 nm. Meanwhile, the absorbance at 600 nm was measured to eliminate the interference of sucrose, and MDA was calculated by the difference between the absorbance at 532 nm and 600 nm.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Extraction Buffer	60 mL	120 mL	4℃
Cell Extraction Buffer	60 mL	120 mL	4℃
Reagent Mix	18 mL	36 mL	4℃

※Before the formal measurement, be sure to take 2-3 samples with large expected differences for pre-determination.

Materials Required but Not Supplied

- Microplate reader or visible spectrophotometer capable of measuring absorbance at 505 nm and 600 nm.
- Water bath, centrifuge
- 96-well plate or micro glass cuvette, precision pipettes, disposable pipette tips
- Deionized water
- Homogenizer (for tissue samples)

Biorbyt Ltd
7 Signet Court, Swann Road, Cambridge, CB5 8LA. United Kingdom
Email: info@biorbyt.com | Phone: +44 (0)1223 859-353 | Fax: +44 (0)1223 280-240

Biorbyt LLC
68 TW Alexander Drive, Durham, NC 27713. United States
Email: info@biorbyt.com | Phone: +1 (415) 906-5211 | Fax: +1 (415) 651-8558

Reagent Preparation

Extraction Buffer: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃.

Cell Extraction Buffer: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃.

Reaction Mix: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃. If there is a precipitate, it can be placed in a 70℃ water bath until the precipitate dissolves.

Sample Preparation

1. Animal or Plant Tissues: Weigh about 0.1 g Tissue and add 1 mL ice-cold Extraction Buffer, homogenize on ice. Centrifuge at 13,000 g for 10 min at 4℃, take the supernatant for further analysis.
2. Cells: Collect 1×10^7 cells. Wash cells with cold PBS, discard the supernatant after centrifugation, add 0.5 mL ice-cold Cell Extraction Buffer and the cells are lysed in an ice bath for 5-10 min, then mix well once every 3 min. Centrifuge at 13,000 g for 10 min at 4℃, take the supernatant for further analysis.
3. Bacteria: Collect 1×10^7 bacteria. Wash bacteria with cold PBS, discard the supernatant after centrifugation, add 0.5 mL ice-cold Extraction Buffer to ultrasonically disrupt the bacteria in ice bath 5 min (power 20% or 200 W, ultrasonic 3 s, interval 7 s, repeat 30 times). Centrifuge at 13,000 g for 10 min at 4℃, take the supernatant for further analysis.
4. Serum, Plasma and Urine: It can be used directly for testing, and if necessary, it is recommended that the sample be diluted to different concentrations before testing.

Note: Fresh samples are recommended. If you cannot perform the assay at the same time, we suggest that you freeze the sample at -80℃. The sample will be stable for at least one month.

Assay Procedure

1. Preheat the microplate reader or visible spectrophotometer for more than 30 min, visible spectrophotometer was returned to zero with deionized water.
2. Add the following reagents to EP Tube:

Reagent	Blank Tube (μL)	Test Tube (μL)
Reaction Mix	300	300
Deionized Water	100	0
Sample	0	100

3. Mix well and incubate in water bath 30 min at 95℃ (cover tightly to prevent moisture loss). Then cool to room temperature in an ice bath. Centrifuge for 10 min at 25℃ at 10,000 g.
4. Transfer 200 μL of the supernatant into a 96-well plate or micro glass cuvette. Measure the absorbance at 532 nm and 600 nm. Calculate $\Delta A=A_{532}-A_{600}$. Calculate $\Delta\Delta A_{Test}=\Delta A_{Test}-\Delta A_{Blank}$ (Only one blank well needs to be detected).

Biorbyt Ltd
7 Signet Court, Swann Road, Cambridge, CB5 8LA. United Kingdom
Email: info@biorbyt.com | Phone: +44 (0)1223 859-353 | Fax: +44 (0)1223 280-240

Biorbyt LLC
68 TW Alexander Drive, Durham, NC 27713. United States
Email: info@biorbyt.com | Phone: +1 (415) 906-5211 | Fax: +1 (415) 651-8558

Note: In order to guarantee the accuracy of experimental results, need to do a pre-experiment with 2-3 samples. If $\Delta\Delta A_{Test}$ is greater than 1.0, the sample can be appropriately diluted with Extraction Buffer, the calculated result multiplied by the dilution factor. If $\Delta\Delta A_{Test}$ is less than 0.001, increase the sample quantity appropriately.

Data Analysis

Note: We provide you with calculation formulae, including the derivation process and final formula. The two are exactly equal. It is suggested that the concise calculation formula in bold is final formula.

A. 96-well plate calculation formula

1. Calculated by protein concentration

MDA (nmol/mg prot)=$[\Delta\Delta A_{Test}\times V_{Reaction\ Total}\div(\varepsilon\times d)\times 10^9]\div(Cpr\times V_{Sample})$=**$51.6\times\Delta\Delta A_{Test}\div Cpr$**

2. Calculated by fresh weight of samples

MDA (nmol/g fresh weight)=$[\Delta\Delta A_{Test}\times V_{Reaction\ Total}\div(\varepsilon\times d)\times 10^9]\div(W\times V_{Sample}\div V_{Sample\ Total})$=**$51.6\times\Delta\Delta A_{Test}\div W$**

3. Calculated by number of cells or bacteria

MDA (nmol/10^4)=$[\Delta\Delta A_{Test}\times V_{Reaction\ Total}\div(\varepsilon\times d)\times 10^9]\div(1{,}000\times V_{Sample}\div V_{Sample\ Total})$=**$0.0258\times\Delta\Delta A_{Test}$**

4. Calculate by volume of liquid sample(serum, plasma and urine)

MDA (nmol/mL)=$[\Delta\Delta A_{Test}\times V_{Reaction\ Total}\div(\varepsilon\times d)\times 10^9]\div V_{Sample}$=**$51.6\times\Delta\Delta A_{Test}$**

Notes: $V_{Reaction\ Total}$: total reaction volume, 4×10^{-4} L; ε: MDA molar extinction coefficient, 155×10^3 L/mol/cm; d: 96-well plate diameter, 0.5 cm; 10^9: 1 mol=1×10^9 nmol; Cpr: sample protein concentration, mg/mL; V_{sample}: sample volume added, 0.1 mL; W: sample weight, g; $V_{Sample\ Total}$: Tissues Extraction Buffer volume added, 1 mL, cells or bacteria sample volume added, 0.5 mL; 1,000: Total number of cells or bacteria, 1×10^7.

B. Microglass cuvette calculation formula

The optical diameter d:0.5 cm in the above calculation formula can be adjusted to d:1 cm for calculation.

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes.

Biorbyt Ltd
7 Signet Court, Swann Road, Cambridge, CB5 8LA. United Kingdom
Email: info@biorbyt.com | Phone: +44 (0)1223 859-353 | Fax: +44 (0)1223 280-240

Biorbyt LLC
68 TW Alexander Drive, Durham, NC 27713. United States
Email: info@biorbyt.com | Phone: +1 (415) 906-5211 | Fax: +1 (415) 651-8558